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SHORT COMMUNICATION

Homogenate extraction technology of camptothecine and hydroxycamptothecin from *Camptotheca acuminata* leaves

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Abstract: Camptothecine (CPT) and hydroxycamptothecin (HCPT), two kinds of anti-cancer alkaloids, were extracted from *Camptotheca acuminata* leaves using homogenate extraction technology under different conditions such as the ratio of material to liquid, ethanol concentration, and homogenate time. The optimum technology parameters for homogenate extraction of CPT and HCPT from *C. acuminata* leaves were determined as homogenate time at 8 min, ethanol concentration at 55% and the ratio of material to liquid at 1:15 (g:mL). By using the optimized parameters, we obtained 0.639‰ extraction rate for CPT and 0.437‰ for HCPT. The extraction yields of CPT and HCPT extracted by homogenating technology were higher than those by other extractive methods, such as ultrasonic, reflux, shaking in water bath. It is concluded that the homogenate extraction technology was an efficient method for extracting CPT and HCPT from *C. acuminata* leaves, with characteristics of less extraction time and high yield.

Keywords: homogenate; *Camptotheca acuminata* leaves; camptothecine; hydroxycamptothecin

Introduction

Camptotheca acuminata Decne. is an indigenous tree in southern China. It produces anti-tumor alkaloids, most notably, camptothecin (CPT). CPT was first reported to possess anti-tumor activity in the 1960s (Kepler et al. 1969). In the 1980s, it was found that the anti-tumor activity of CPT was due to its ability to inhibit topoisomerase I, an enzyme involved in DNA replication. In addition, a number of CPT derivatives were also found to be an effective medicine for curing colon and breast cancers in human clinical trials. Naturally occurring CPT derivatives were reportedly present in *C. acuminata*. Among these derivatives, hydroxycamptothecin (HCPT) has more potent and less toxic (Zhang et al. 1998; Wiedenfeld et al. 1997) and exhibits a strong apoptosis-inducing effect on human hepatoma Hep G2cells (Zhang et al. 1999; Zhang et al. 2000).

Many extraction methods including ultrasonic extraction, reflux extraction and shaking extraction were once used to extract CPT and HCPT (Fulzele et al. 2005). Nevertheless, all of the methods above suffered from some limitations such as time consuming and low yield. Homogenate extraction is basically to put material and solvent into the homogenate extraction equipment, then simultaneously making material smashing and extracting of chemical compositions by mechanism and fluid cutting action. This method has been documented to be effective in extracting protein, amino acid, terpenoids, etc (Holford et al. 2004; Zu et al. 2005). However, limited information is available on homogenate extraction technology for alkaloids. In the present study, we examined the efficiency of homogenate extraction of CPT and HCPT from *C. acuminata* leaves. The effects of various extraction parameters such as concentration of extraction solvent, ratio of material to liquid and extraction time on the extraction yield were investigated. Furthermore, homogenate extraction method was compared with other methods including ultrasonic extraction, reflux extraction and shaking extraction.

Materials and methods

Plant materials and reagents

C. acuminate leaves were collected from Jintang County, Sichuan Province, China and identified by Professor Nie Shaoquan, Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin, China. Ethanol was of analytical grade (Beijing Chemical Reagents Company, China). Acetonitrile was of high performance liquid chromatographic (HPLC) grade (Tedia Company, USA). Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, USA). Camptothecin (CPT) and hydroxycamptothecin (HCPT) standard were purchased from Sigma Company.

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Apparatus

The homogenate extraction was carried out by Homogenate Extraction Equipment in the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, China. Ultrasonic Bath (KQ-250DB) was provided by Kunshan Ultrasonic Instrument Ltd, China. Shaking extraction was carried out in a Reciprocating Water Bath Shaking Incubator (ZD-85, Jiangsu Jintan Eltong Electric Corp, China). Rotary evaporator (RE5203) was provided by Shanghai Yarong Biochemistry Instrument Factory. Ltd. All analyses were performed on high performance liquid chromatography (Jasco Corporation, Japan).

Methods

Optimization of homogenate extraction

Considering the cost and safety of solvent, we use ethanol as extraction solvent. The dried leaves of *C. acuminata* were cleaned. We put 30-g dried leaves with different volume and concentration of ethanol in homogenate extraction equipment for homogenate extraction for different time at 20°C. For each sample, there were three replicates. All the data were presented using an average with standard deviation.

Comparisons of different extraction methods

For ultrasonic extraction, 30-g *C. acuminata* leaves (leaf powder dried under 60°C, 60 mesh sifter) with 450 mL of 55% ethanol was put in an ultrasonic bath at 45°C for one hour. For reflux extraction, 30-g leaves (leaf powder dried under 60°C, 60 mesh sifter) was heated with 450 mL of 55% ethanol in a flask for 6 h at 90°C. Shaking extraction was conducted by puting 30 g of leaves (leaf powder dried under 60°C, 60 mesh sifter) with 450 mL of 55% ethanol in a reciprocating water bath shaking incubator for 12 hour at 50°C. For homogenate extraction, the leaves (30 g) was extracted directly with 450 mL of 55% ethanol in homogenate extraction equipment for 8 min at 20°C. Each extraction was repeated for three times.

High performance liquid chromatographic (HPLC) *conditions*

The analytical HPLC system consisted of a Jasco high-performance liquid chromatography coupled with a UV–vis detector (MD-910 Jasco, Japan). The separation process was achieved on a HIQ SIL C18V reversed-phase column (ø4.6 mm × 250 mm KYA TECH Corporation, Japan) packed with 5-μm diameter particles, and the mobile phase consisted of acetonitrile –water (30:70, v/v). Flow rate and injection volume were 1.0 mL/min and 10 μL, respectively.

The monitoring wavelength was 254 nm in 0-8 min. After 8 min the wavelength was shifted to be 266 nm. The extract was collected and filtered. The filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried extract was dissolved in the mobile phase. After filtering through a filter paper with a 0.45-μm membrane filter (Millipore), the extract was injected directly. All chromatographic operations were carried out at ambient temperature.

Results and discussion

Effect of the ratio of material to liquid on extraction yield of CPT and HCPT

The effect of different ratio of material to liquid on extraction yield of CPT and HCPT was detected at 55% ethanol concentration, 8-min homogenate time and 20°C. Results show that extraction yield of CPT and HCPT increases with the increase of ratio of material to liquid (Fig. 1). When the ratio of material to liquid was lower than 1:10 (g: mL), CPT and HCPT could not be extracted completely. The extraction yield of CPT and HCPT had a little increase at the ratio of material to liquid of 1:15 to 1:25. For large-scale production, higher ratio of material to liquid needs to be coordinated with more cost. Hence, the ratio of material to liquid (1:15 (g:mL)) is selected as an economical extraction parameter.

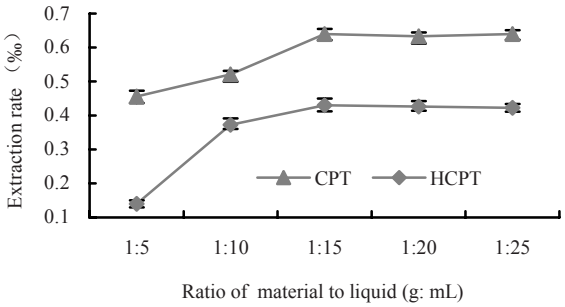

Fig. 1 Effect of ratio of material to liquid on extraction yields of camptothecin (CPT) and hydroxycamptothecin (HCPT) from *Camptotheca acuminata* Leaves

Effect of ethanol concentration on extraction yield of CPT and HCPT

The effect of different ethanol concentrations on extraction yield of CPT and HCPT was detected at ratio of material to liquid of 1:15 (g:mL), 8-min homogenate time and 20°C. Results showed that when ethanol concentration was lower than 55%, extraction yield of CPT and HCPT increased with the increase of ethanol concentration. Extraction yield of CPT and HCPT reached the highest value at ethanol concentration of 55%, and then decreased slowly with the increase of ethanol concentration (Fig. 2). Therefore, we concluded that 55% ethanol concentration was the best concentration for extracting CPT and HCPT.

Effect of homogenate time on extraction yield of CPT and HCPT

The effect of homogenate time on extraction yield was detected at 55% ethanol concentration, 1:15 (g:mL) of the ratio of material to liquid and 20°C. The extraction yield increased with homogenate time from 2 to 16 min (Fig. 3). Results demonstrated that extraction yield of CPT and HCPT was nearly the highest at the special time point of 8 min. After that, the increasing scale of CPT and HCPT extraction yield was smaller. The material (*C.*

acuminata leaves) would be very tiny if we extended the homogenate time, and the tiny material would increase the difficulty to the next separation process. Therefore, according to our experiment results, the homogenate time of 8 min was enough for extraction of CPT and HCPT.

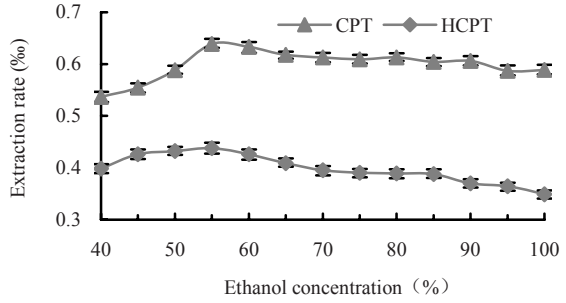

Fig. 2 Effect of ethanol concentration on extraction yields of camptothecin (CPT) and hydroxycamptothecin (HCPT) from *Camptotheca acuminata* Leaves

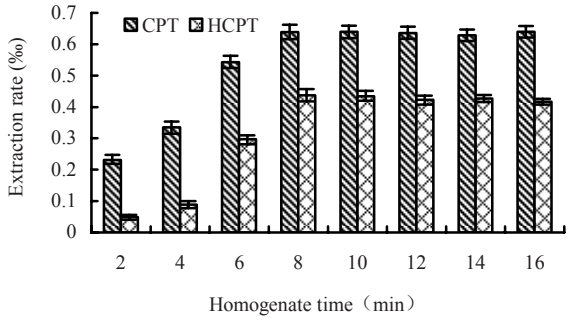

Fig. 3 Effect of homogenate time on extraction yields of camptothecin (CPT) and hydroxycamptothecin (HCPT) from *Camptotheca acuminata* Leaves

Comparisons of extraction yield of CPT and HCPT by different methods

The extraction rates of CPT and HCPT obtained by different extraction methods are shown in Table 1. The extraction yields of CPT and HCPT extracted by homogenating technology were higher than those by other extractive methods, such as ultrasonic, reflux, shaking in water bath (Table 1).

Table 1. Comparisons of different methods on extraction yield of camptothecin (CPT) and hydroxycamptothecin (HCPT) from *Camptotheca acuminata* Leaves （n=3）

Extraction methods	Extraction time (h)	Extraction temperature (°C)	Extraction rate(‰)	
			CPT	HCPT
Ultrasonic	1	45	0.623	0.429
Reflux	6	90	0.628	0.430
Shaking	12	50	0.435	0.262
Homogenate	0.133	20	0.639	0.437

The homogenate extracting technology has a characteristic of time saving and high efficiency. This technology has been used in enlarged production of 20.0-kg material in Engineering Research Center of Forestry Bio-preparation, Ministry of Education, Northeast Forestry University, and acquired prospective results.

Conclusion

In this paper, the homogenate extraction technology was used to extract CPT and HCPT. The optimal parameters were found as follows: homogenate time at 8 min, 55% ethanol and a ratio of material to liquid of 1:15 (g:mL). Under these extraction conditions, the extraction rate of CPT and HCPT is 0.639‰ and 0.437‰, respectively, which is higher than that by other extractive methods. The results show that homogenate extraction technology also has many advantages, such as time saving, low temperature and high efficiency. Our study desmonstrates that homogenate extraction is a reliable and fast method for effective extraction of CPT and HCPT from *C. acuminata* leaves.

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